

AGAROSE GEL ELECTROPHORESIS OF DNA

Why would anyone want to study DNA? Scientists have learned that the incredible amount of information stored in DNA can answer many questions and solve problems, which affect people daily. For example, the forensic analysis of DNA can help convict (or free) suspected criminals, solve cases of poaching of endangered species, and determine which species of salmon is migrating up the Columbia River. The ability to identify specific bacterial strains using a DNA profile allows meat to be tested for the presence of harmful strains of *E. coli*, preventing fatal food poisoning. People can be tested for the presence of harmful genes, and someday those genes will be repaired through gene therapy. Biotechnology uses genes to make products humans need for fighting disease. These are all cases in which the analysis of DNA is required.

You will be working on the problem of conserving a species with the help of biotechnology and the analysis of genomic DNA. The species of organism involved is *Loxodonta africana* or the African elephant. Because it is difficult to obtain this elephant DNA for classroom use, you will be using the DNA of a virus named lambda. Since all DNA is composed of the same four nucleotides and lambda DNA is a well-known and readily accessible form of DNA, it provides an excellent model for you to use in this lab. You will be working with a biotechnology technique called gel electrophoresis to analyze your genomic DNA.

Pieces of genomic DNA are too big to analyze on the kind of agarose gel that we (and many other research scientists) are using. Proteins called restriction enzymes are used to cut DNA at specific DNA sequences creating smaller fragments that can be visualized on an agarose gel. Each restriction enzyme cuts DNA at a particular nucleotide sequence acting like molecular scissors. Keep in mind that DNA from any organism can be studied using these same concepts and technologies. The analysis of DNA by gel electrophoresis is a necessary step in many experiments using DNA.

In your lab you will find that some samples (those from known locations) have already been cut by a restriction enzyme and are ready for analysis by electrophoresis. One sample, the model ivory sample, you will have to cut yourself. This sample of genomic DNA will be cut by the restriction enzyme BamHI. BamHI, cuts DNA at the sequence



This sequence of DNA is called the **recognition site** for BamHI

Whenever BamHI encounters this sequence in a strand of DNA, it cuts, breaking the sugar-phosphate backbone between the two Gs on both strands of the DNA. Each enzyme cuts DNA in a predictable and reproducible manner. Since the early 1970s, hundreds of restriction enzymes have been discovered and catalogued according to their recognition sites. Thus, it is possible to choose from a library of these enzymes to cut DNA at chosen sequences.

Restriction enzymes are produced by, and derived from, various bacteria. Their funny names come about from the following rules:

	T
L	

1

1

L

1

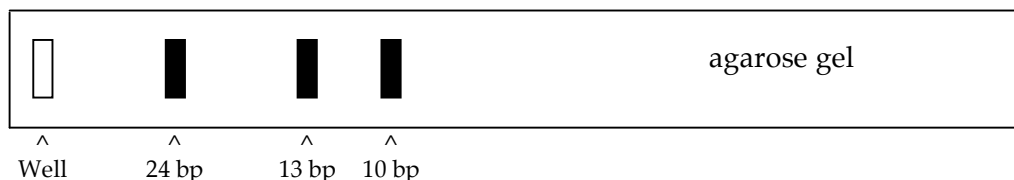
The following diagram is an example of the restriction digest followed by electrophoresis:

5' Bam HI 3' Bam HI 3'

G GATAG \ GATCCCACACACAGAAAGCGATCG \ GATCCATGATAGC

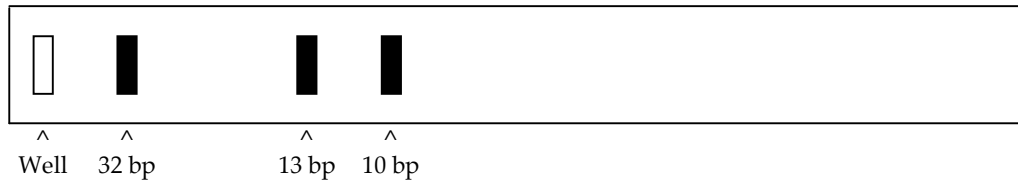
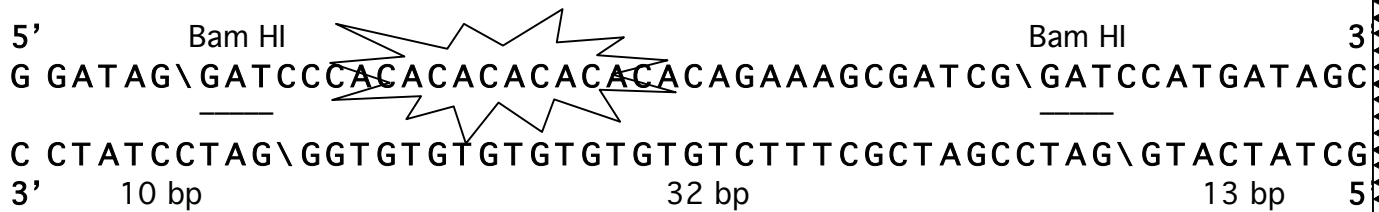
C CTATCCTAG \ GGTGTGTGTCTTTCGCTAGCCTAG \ GTACTATCG

3' 10 bp 24 bp 13 bp 5'



One method deals with finding sites that are polymorphic or variable in the organism you are studying. These polymorphic areas usually contain either insertions, deletions or substitutions of nucleotides (A, C, G or T). They can also demonstrate a tandem repeat (AGAGAGAGAG, etc.) These polymorphisms are often called mutations and usually occur in an area of the DNA that does not code for a necessary function of the organism but is passed down to the offspring. See diagram below:

ELEPHANT GROUP B



Thus, different mutations can cause a difference in the fragment lengths of the DNA and this difference is detectable on the gel electrophoresis-banding pattern. Since it is inheritable, offspring can be traced by finding the patterns in family lines.

What do you think would happen to the length of DNA fragments and/or the banding pattern if a mutation occurred at the recognition site of a restriction enzyme? Use the above diagram to explain.

RFLP ANALYSIS OF DNA LABORATORY

BIG PICTURE

You will be working with an essential research method widely used in genetics, conservation biology, and forensics. The lab is divided into three sections.

- ↳ Part 1: Your lab team will perform an enzyme digest of DNA from confiscated ivory to identify the origin of that ivory.
- ↳ Part 2: Your lab team will perform gel electrophoresis of pre-digested samples. You will compare the data to a database of genomic DNA. If you discover new data, you will contribute the data to the database.
- ↳ Part 3: Your lab team will analyze gel data from the actual research lab to compare to your samples from part A.

KEY CONCEPTS

When you have completed this lab you should be able to:

- Understand the physical properties of DNA molecules that allow separation by agarose gel electrophoresis.
- Understand how the use of biotechnology and the application of the DNA fingerprint technique (RFLP) can be used to conserve a species.
- Understand the techniques of micropipetting, sample preparation and electrophoresis as tools for gathering, analyzing, and interpreting data.
- Understand how scientists might use a database in scientific research and in conservation of a species.

STUFF TO KNOW BEFORE YOU GO

- ↳ **Important:** The restriction enzymes are in very viscous (thick) solutions because they contain 50% glycerol. To pipet these tiny volumes like a “pro,” first depress the plunger, then place just the end of the pipet tip into the enzyme solution and release the plunger slowly. When adding the enzyme to the sample tube, place the tip into the droplet containing water, buffer, and DNA. Slowly push out the enzyme; then pipet in and out slowly a few times to rinse all of the enzyme out of the tip.
- ↳ **Note:** One unit (U) of activity is usually defined as the amount of enzyme required to digest 1 µg of lambda DNA to completion in 1 hour in the preferred enzyme buffer at the optimal temperature for that enzyme (usually 37°C). Whew!

**** It's important to use the right buffer to provide the best conditions for each enzyme. The enzymes have personalities and work best under different salt conditions. The manufacturer we use calls its buffers REact®. You will be using 10X REact 3® in this lab investigation.**

PART 1: PREPARING THE GEL AND DNA SAMPLES

Your team may want to divide up these responsibilities or you may choose to work on each task as a group. If you choose to work as a group, **MOVE QUICKLY FROM ONE TASK TO THE NEXT.** Time is short. This must be set up today.

1. Prepare a 0.7% agarose gel.

☞ See “How to Make a Carolina Blu gel.” Your teacher will help you with this step.

☞ When the gel is cool:

- carefully remove the comb from the gel.
- store the gel in buffer overnight.
- See you teacher for instructions on where to store your gel!

2. Prepare the Enzyme digest of the ivory DNA: Tube #6 (see chart 1)

- ☞ Keep the restriction enzyme stocks on ice all the time
- ☞ Add the reagents in the order listed, adding the enzyme last.
- ☞ Check off ✓ each ingredient on the chart as it is added.
- ☞ Mix tube contents gently, centrifuge 3 seconds.
- ☞ Incubate at 37° for at least 30 minutes or longer.

3. Prepare the pre-digested samples: Tubes 1-5 (see chart 1)

- ☞ Label tubes with your team’s # and what is in each tube.
- ☞ Add reagents in the order given.
- ☞ Check off ✓ each ingredient on the chart as it is added.
- ☞ Mix all tube contents gently, centrifuge 3 seconds.
- ☞ Place on ice.

4. Place all tubes (1-6) in the freezer until the next lab period. See your instructor for where you will place these tubes

Chart 1

TUBE	1 Ladder	2 Uncut genomic DNA	3 Serengeti	4 S.Luangwa	5 Etosha	6 Ivory Sample
(color or label)	_____	_____	_____	_____	_____	_____
Sterile distilled H ₂ O	10.5μl	10.5μl	10.5μl	10.5μl	10.5μl	8.5μl
10X REact 3 restriction enzyme buffer	1.5μl	1.5μl	1.5μl	1.5μl	1.5μl	1.5μl
Serengeti sample	—	—	3μl	—	—	—
S. Luangwa sample	—	—	—	3μl	—	—
Etosha sample	—	—	—	—	3μl	—
Uncut genomic DNA	—	3μl	—	—	—	—
Ladder (Marker)	3μl	—	—	—	—	—
Ivory DNA sample	—	—	—	—	—	4μl
Restriction enzyme BamHI	—	—	—	—	—	1μl

PART 2: RUNNING THE GEL

1. Set up the gel.

- ✎ Obtain 125ml of 1X TAE buffer containing Carolina Blu-1.
 - ✎ If your gel was made earlier, place the gel into the gel box, sample wells near the negative (black) electrode and top side of the gel facing up. If your gel was poured today, add a little buffer around the comb, and gently remove the comb and dams.
 - ✎ Add buffer with Carolina Blu until the buffer level is about 2mm above the top of the gel.
 - ✎ Put the gel box near the power supply before loading samples.
 - ✎ Once you start loading samples in the next step, you should avoid shaking or moving the gel box. (Optional: check pH at each electrode.)
2. **Remove tubes 1-6 from the freezer.** Place them on the lab bench until they thaw.
3. **Then add 3 μ l Sample Loading Buffer** to each. If necessary, centrifuge again.

TUBE	1 Ladder	2 Uncut genomic DNA	3 Serengeti	4 S.Luangwa	5 Etosha	6 Ivory Sample
(COLOR OR LABEL)						
Sample Loading Buffer(microcentrifuge tube of blue soln.)	3 μ l	3 μ l	3 μ l	3 μ l	3 μ l	3 μ l
Total Volume	18 μ l	18 μ l	18 μ l	18 μ l	18 μ l	18 μ l

4. **Load 15 μ l of your samples** into the wells.
- ✎ On your record sheet, or in our journal indicate which sample is in each well.
 - ✎ When all the samples are loaded, close the lid on the gel box.
5. **Beginning Gel electrophoresis**
- ✎ **Check that the power supply is turned off and the voltage is turned all the way down.**
 - ✎ Then attach the electrodes of the gel box to the power supply, making sure that the red lead connects the positive (+) terminals and the black lead connects the negative (—) terminals.
 - ✎ Usually two student teams will be sharing one power supply. If one group starts their run first, just turn off the power supply while connecting the second gel box. Adjust the power supply to ~100 volts.
6. **Record the following information in your journal:**
- Start time _____ Total electrophoresis time _____
- Voltage _____ Current (may change during the run) _____
7. **Running the gel**
- ✎ Electrophorese for at least 5-10 min to make sure the samples have entered the gel from the wells. Your teacher will tell you if this is the stopping point for today or how long to run your gel.
 - ✎ The ideal electrophoresis time will be 45 min at 100 volts. Less time will result in poor separation of the DNA bands.

- ✦ You can monitor the general progress of the electrophoresis by watching the tracking dyes included in the sample loading buffer, bromophenol blue (at this pH it's purple) and xylene cyanol. It's best to run the gels until the bromophenol blue has moved about 2/3 of the way through the gel (or halfway if time is short).
8. **Termination of electrophoresis:**
- ✦ After 45 min or at the time your instructor indicates, **turn off the power supply.**
 - ✦ **Then** detach the electrical leads (optional: record the pH at each end of the gel box).
 - ✦ Note the positions of the tracking dyes on your record sheet.
 - ✦ Carefully transfer the gel to a staining dish bearing your group's names or initials.
 - ✦ Stain the gel with Carolina Blu-2, as described on the blue "How to make a Carolina Blu gel" sheet.
9. **Clean up** your lab station, dispose of designated materials as directed. Wash your hands.



PART 3 – CALCULATION OF RFLP LENGTHS USING BANDING PATTERNS AND SIZE APPROXIMATION

- ☐ Obtain a small acetate sheet for each member of your group.
- ☐ Place the gel on a light source.
- ☐ Carefully place the acetate over the top of the gel.
- ☐ Trace the outline of the wells.
- ☐ Number the wells.
- ☐ Trace the DNA fragment lengths as accurately as possible.
- ☐ Tape your completed acetate sheet into your journal.
- ☐ Label the marker fragments with the number of kilobase pairs they represent.
- ☐ Locate an unknown fragment you are trying to measure. Use a straight edge or ruler to compare the location of the unknown band to the known marker bands.
- ☐ Estimate the size of the unknown band based on the comparative location to the nearest markers.
- ☐ Share data with your table partners and calculate the average base pair length of each unknown fragment.
- ☐ Record the data in your Data Submission Form (DS571).



ANALYSIS OF RFLP DATA BY SIZE ESTIMATION

BACKGROUND INFORMATION FOR ANALYSIS OF DATA

MATERIALS: Ruler or straight edge and a copy of the Comstock data

When we start working with DNA, one of the first things we can learn about it is the size of the DNA. Size is such a basic piece of information that unknown DNA fragments are often first named just by their size.

It's possible to make a reasonable guess of the sizes of bands on a gel by comparing them by eye to a marker band. Since we know what size the marker's bands are supposed to be, when the band whose size we're trying to determine lines up pretty close, we've probably got a band of similar size. A much more accurate method, however, is to graph our results on semi-log paper.

The marker DNA gives us a ruler for determining the size of our unknowns. We first note what the markers have done in our gel. We use that information to determine the size of our unknown DNA fragments. In a way, we're making our own unique ruler that is accurate for this gel only.

Say we have a very simple gel. We've run a marker that gives us three bands, at 20kb (kilobases, or thousand bases), 5.3 kb and 2.9 kb. We've also run an unknown sample. Here's what the gel looks like: We can make a safe guess that the lower marker band is the 2.9kb band the next one is 5.3kb.

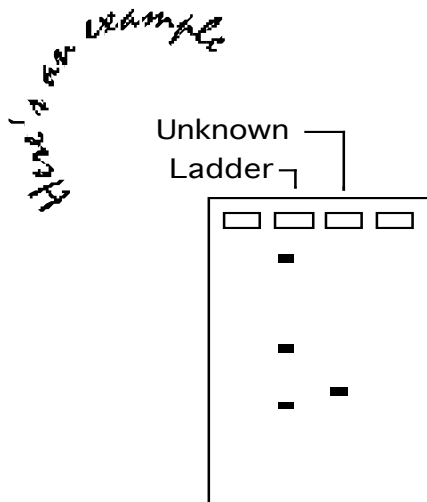


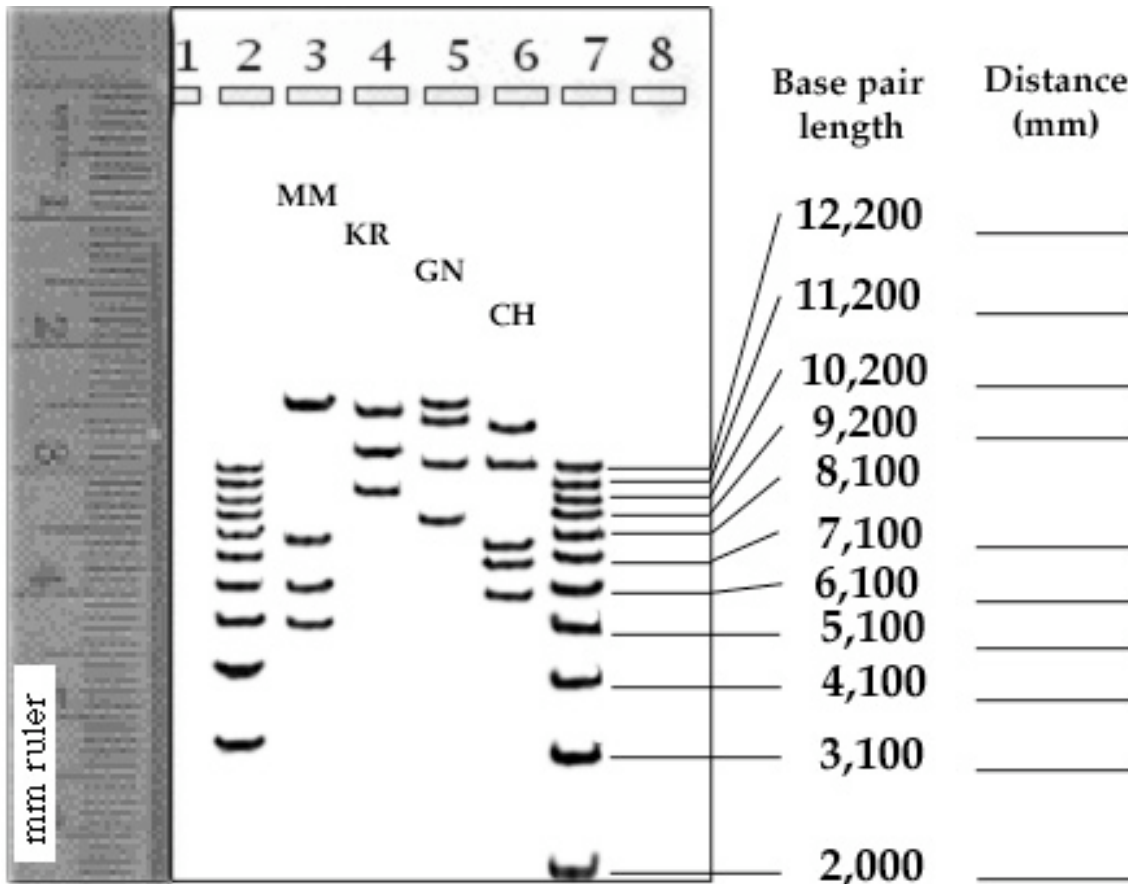
Figure 1. Gel showing DNA fragment bands from an unknown and ladder (marker) sample.

Since all DNA should migrate in the same way (i.e., assuming the same conformation or shape of the DNA and uniform charge), we can eyeball the size of the unknown band. It looks like it's between 3.5kb and 4kb. This is just a rough guess, though. That guess might be good enough, but what if we need a more accurate size estimate? We do a semi-log plot!

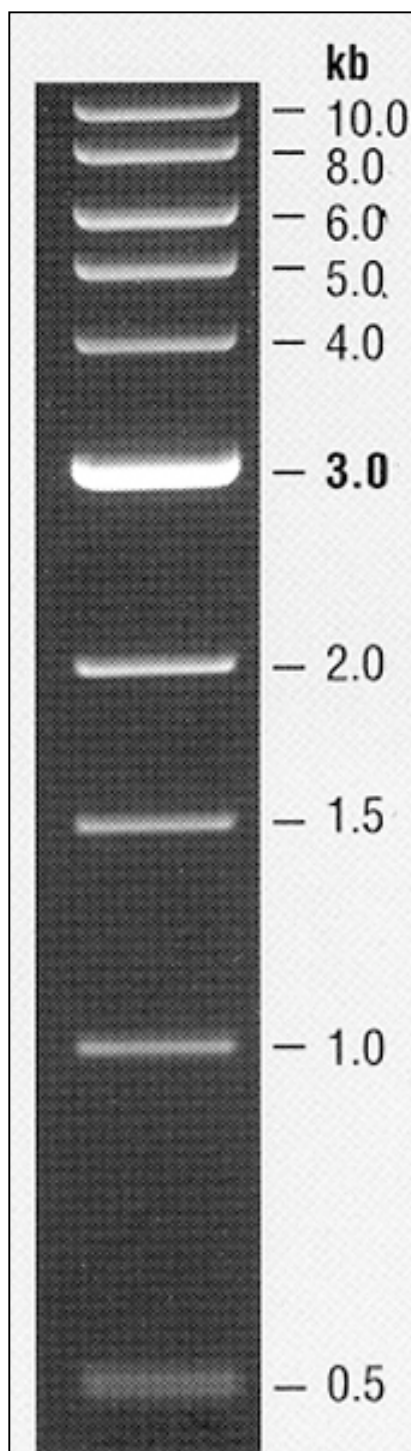
TO ANALYZE THE COMSTOCK GEL, PLEASE DO THE FOLLOWING:

Using the photocopy below of the gel from Kenine Comstock's lab, an estimation of the size (base pair length) of unknown bands on this gel can be determined by comparing the unknown bands to the bands of the known marker or ladder. The known base pair lengths of the marker are written along the right side of the gel and their corresponding band is indicated by a connecting line.

1. Choose an unknown band you want to estimate in base pair length,
2. Find the bands on the marker or ladder that are just above and just below the unknown band you have chosen. Note the length of each of these known ladder bands.
3. Based on the length of your known bands and the distance your unknown band is from each, estimate the size of your unknown band.
4. Compare the estimated length of your unknown band to that of others in your lab team, record the average estimated length of all team members on your data submission form.



***1 KB LADDER FOR
ELEPHANT RFLP ANALYSIS***



Additional information can be obtained from New England Biolabs at:
<http://www.neb.com/nebecomm/products/productN3232.asp>

TO ANALYZE YOUR OWN GEL, PLEASE DO THE FOLLOWING:

1. Record the length of each of the fragments of your known ladder or marker next to the appropriate band on the acetate sheet or drawing of your gel. You may want to add a line indicating what base pair length corresponds to each fragment in the marker or ladder, as was done for the Comstock gel data.
2. Repeat steps 2 through 4 above in order to identify unknowns based on the known ladder or marker.
3. Record the estimated lengths of the unknown bands from your gel on the Data Submission Form (DSF).
4. Compare the number and size of fragments from the ivory DNA to that of the Comstock gel database as well as to the samples you ran on your gel.
5. Record the most likely location or source of your ivory, based on DNA fingerprinting or banding patterns (fragment numbers and sizes) observed on your DSF.

DIRECTIONS FOR DATA SUBMISSION DS571

- Record the national park for the samples from Dr. Comstock's laboratory and the base-pair lengths of the RFLP fragments from Table I in list form in the upper portion of the handout called 'Data Submission Sheet.'
- Use the map of Africa you are given to find and record on the data submission sheet the countries in which the four parks are located.
- Using information from the teacher's overhead transparency, record the vegetation regime that prevails at each park (forest or savannah) on the Data Submission Form.

QUESTIONS TO FOLLOW ANALYSIS OF DATA

1. What are the two ways you have to compare RFLP data? What are the advantages and disadvantages of each? Which do you prefer?

2. Elephant populations from the four national parks represented in Dr. Comstock's data appear to have different RFLP patterns? Does this surprise you, or would you expect this? Why?

3. Why do you think that the RFLP patterns seem to be different between parks, but the same within parks?



4. Can you imagine situations in which more than one RFLP pattern might be found in a single park? How about situations in which one RFLP pattern might be found in more than one park?

5. Is knowing the RFLP pattern from one elephant in a park enough to conclude that all elephants in the park share the same pattern? Are two elephants enough? Three? How many would you test to decide that only one RFLP pattern is found in each park?

QUESTIONS ABOUT ELEPHANT POPULATIONS AT RISK

1. What risks to their survival do you think African elephants face today? Are all of the risks due to humans? What non-human risks might affect elephants' survival?

2. Hunting for ivory is not the only human activity that threatens the African elephant's survival. Clearing land for farming and harvesting trees for decorative and insect-resistant lumber both affect elephant habitat. Which two populations of African elephants from your set of Dr. Comstock's samples are most at risk from logging and agricultural activity?

3. Do you think the risks for a park elephant in Africa are different from country to country?



PART 3 – CALCULATION OF RFLP LENGTHS USING SEMI-LOG PLOT

- ❑ Remove your gel from the staining tray.
- ❑ Place gel carefully into a small sealed bag (or clear plastic wrap can be used).
- ❑ Obtain a small acetate sheet for each member of your group.
- ❑ Place the gel on a light source.
- ❑ Carefully place the acetate over the top of the gel.
- ❑ Trace the outline of the wells.
- ❑ Number the wells.
- ❑ Trace the DNA fragment lengths as accurately as possible.
- ❑ Tape your completed acetate sheet into your journal.
- ❑ Plot the ladder as a standard curve using the following steps:
 - ❑ Label the marker bands by size.
 - ❑ Measure the distance that each band has traveled.
 - ❑ Draw the scales for distance and band length on your semi-log graph paper.
 - ❑ Plot the location of each band (distance traveled and size).
 - ❑ Draw a straight line passing as close as possible to all the data points.
- ❑ Based on the distance the unknown band has traveled, estimate its size using the 'standard' line drawn from the marker data.
- ❑ Place the data on the data table provided or in your journal in the table that you have created.
- ❑ Obtain the "Analysis of the unknown genomic DNA and Comstock Data Base" (DS571) **Question sheet** for the next step.

ANALYSIS OF RFLP DATA BY SEMI-LOG PLOT

BACKGROUND INFORMATION FOR ANALYSIS OF DATA

You will learn how to quantify RFLP data from DNA samples separated on an agarose gel by calculating base-pair length from a standard line that you have generated from a sample (the ladder or marker) whose fragment lengths are already known. You will then apply the same process to data from Dr. Comstock's laboratory to characterize the RFLP pattern for elephants from four national parks in Africa.

MATERIALS:

lab handout	millimeter ruler (optional)
semi-log paper	pencil
map of Africa	

When we start working with DNA, one of the first things we can learn about it is the size of the DNA. Size is such a basic piece of information that unknown DNA fragments are often first named just by their size.

It's possible to make a reasonable guess of the sizes of bands on a gel by comparing them by eye to a marker band. Since we know what size the marker's bands are supposed to be, when the band whose size we're trying to determine lines up pretty close, we've probably got a band of similar size.

A much more accurate method, however, is to graph our results on semi-log paper. Because of the wide range of DNA sizes that are resolved on our gels, we use a logarithmic scale on the vertical, or y-axis so we can fit in all the different sizes. On the horizontal, or x-axis, we plot the distance each of those bands has traveled from the wells (in centimeters or millimeters). This is where the name semi-log comes from: semi means half, and log is for logarithmic. One of the two (or half) of the axes is logarithmic.

The marker DNA gives us a ruler for determining the size of our unknowns. We first plot what the markers have done in our gel. We use that information to determine the sizes of our unknown DNA fragments. In a way, we're making our own unique ruler that is accurate for this gel only.

Say we have a very simple gel. We've run a marker that gives us three bands, at 20kb (kilobases, or thousand bases), 5.3 kb and 2.9 kb. We've also run an unknown sample. We can make a safe guess that the lower marker band is the 2.9kb band (why do we know that?), the next one is 5.3kb and that the upper one is 20kb. Here's what the gel looks like:

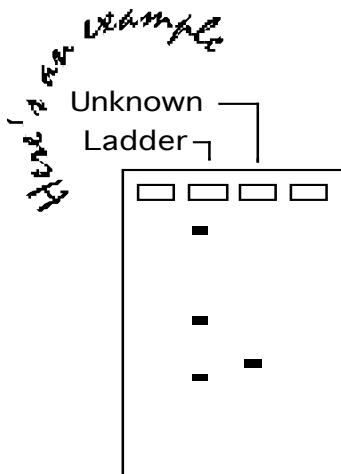


Figure 1. Gel showing DNA fragment bands from an unknown and ladder (marker) sample.

Since all DNA should migrate in the same way (i.e., assuming the same conformation (shape) of the DNA and uniform charge), we can eyeball the size of the unknown band. It looks like it's between 3.5kb and 4kb. This is just a rough guess, though. That guess might be good enough, but what if we need a more accurate size estimate? We do a semi-log plot.

We first measure how far our bands have traveled from the wells. We can measure either from the center of the wells to the center of the bands or from the lower edges of the wells to the lower edges of the bands. It doesn't matter but we have to be consistent. Our 20kb marker band is 18 mm from the well, 5.3kb band is 62 mm from the well, and the 2.9kb band is 75 mm. See Figure 2 for the actual semi-log plot of our results.

Mark a scale on the horizontal, X-axis which can contain these three sizes, plus the starting point of zero. Next, look at the vertical, Y-axis. The scale can be confusing. Each line, marked 1 or 10, where the lines seem to have collapsed represents a 10^1 , or a ten-fold increase. Zero is not on the scale. So, the bottom one is 10^2 , or 100. The next is 10^3 , or 1000. Next is 10^4 or 10,000, and the final one on this scale, marked 10, is 10^5 , or 100,000 base pairs. If we look at the example, and play around with figuring out where a number should go, it'll start making sense.

Now we need to draw a straight line passing as near as possible to all of the points indicating our markers. At the ends of our line, the line may curve. That's fine and is a result of very large or small fragments moving very slowly or very fast. See the example of an actual semi-log plot from a gel with many fragments (Figure 3).

All we know for sure about our unknown band is how far it traveled, so we measure that, just as we did for the marker bands. Our unknown band traveled 72 millimeters. Starting on your semi-log plot at the distance traveled (x axis, 72 mm), move straight up until you intersect with your drawn line. Now go straight left until you hit the y axis, the size scale. Read the size of your unknown band. You should come up with 3,500 or 3.5kb. You've now made a fairly accurate estimate of how large that unknown band is.

To review:

- 1) Identify the marker bands by size.
- 2) Measure the distance that each band has traveled.
- 3) Draw the scales for distance and band length on your semi-log graph paper.
- 4) Plot the location of each band (distance traveled and size).
- 5) Draw a straight line passing as close as possible to all the data points.
- 6) Based on the distance the unknown band has traveled, estimate its size using the 'standard' line drawn from the marker data.

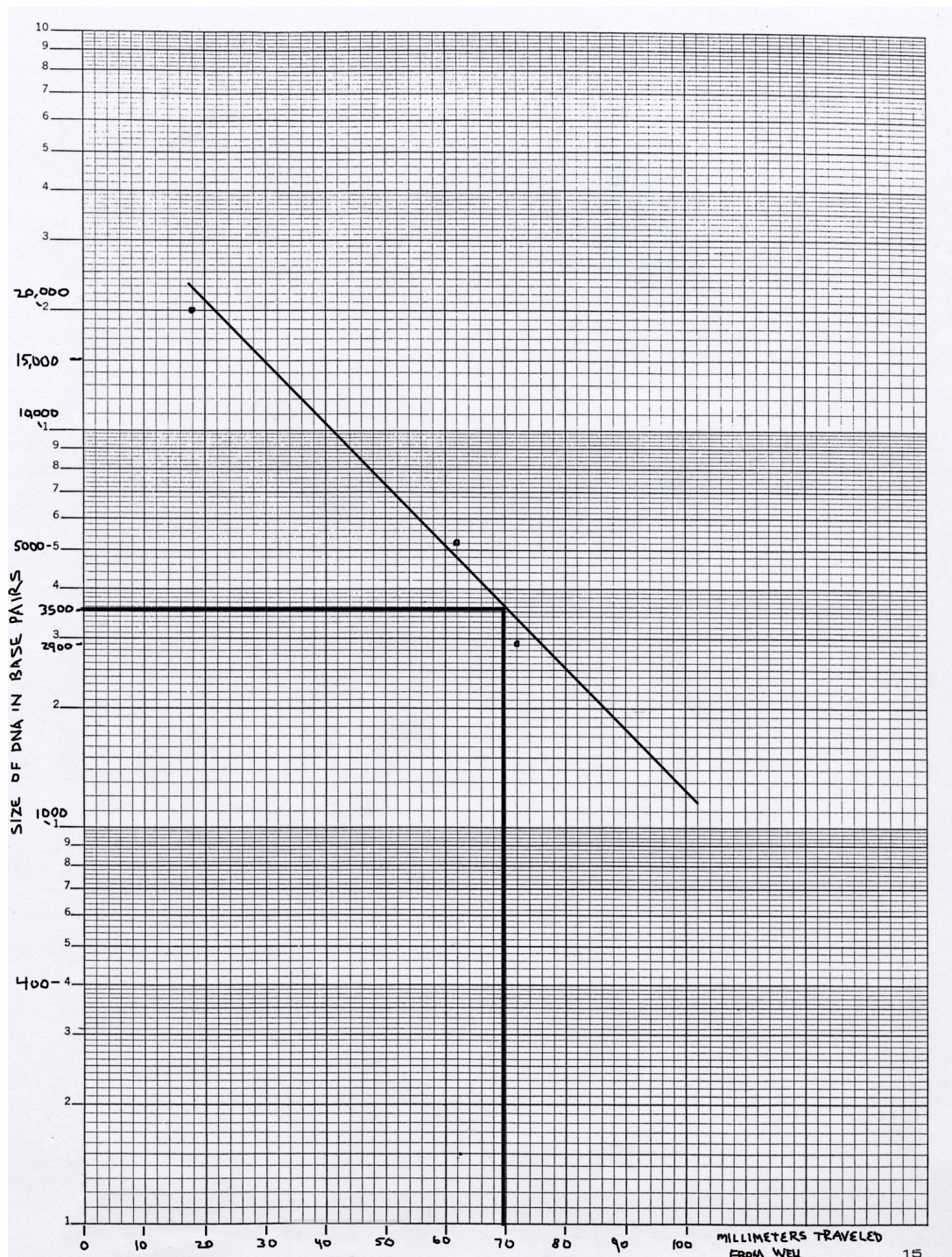


Figure 2

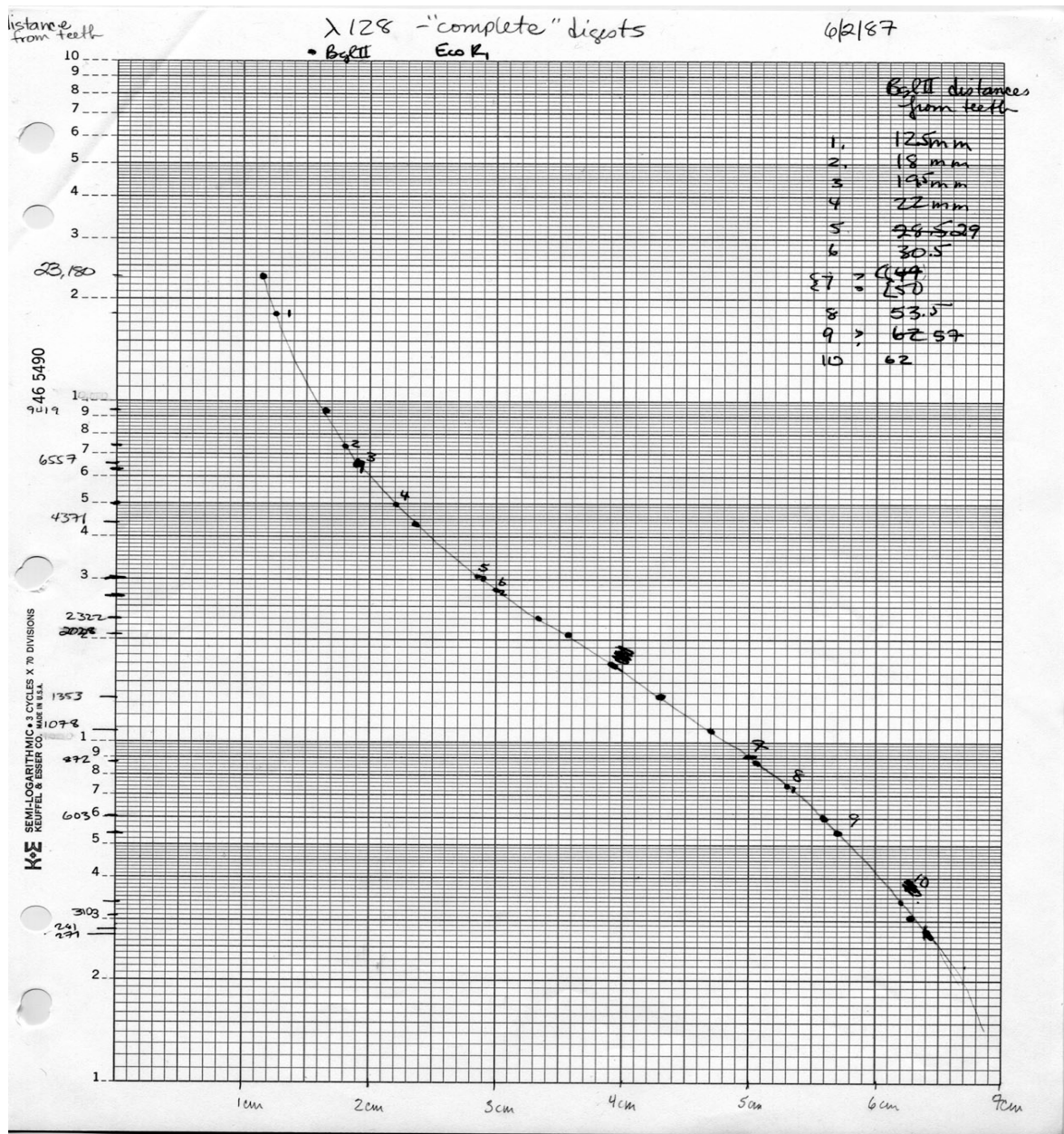
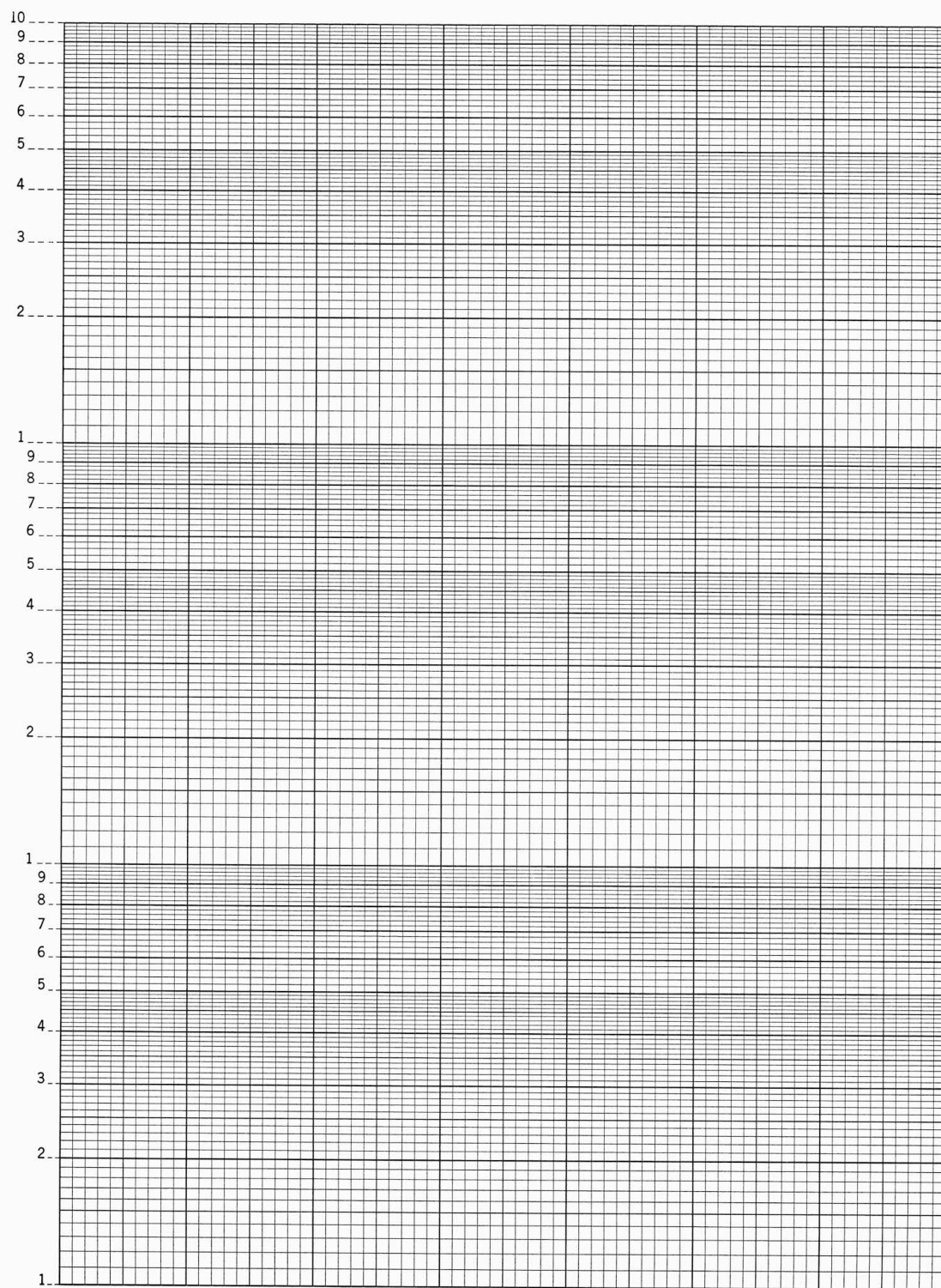


Figure 3 – example of semi-log plot of a standard curve



TO ANALYZE THE COMSTOCK GEL, PLEASE DO THE FOLLOWING:

Using the photocopy below of the gel from Kenine Comstock's lab, create a semi-log plot to determine the size (base pair length) of the unknown bands. The known base pair lengths of the marker are written along the right side of the gel and their corresponding band is indicated by a connecting line.

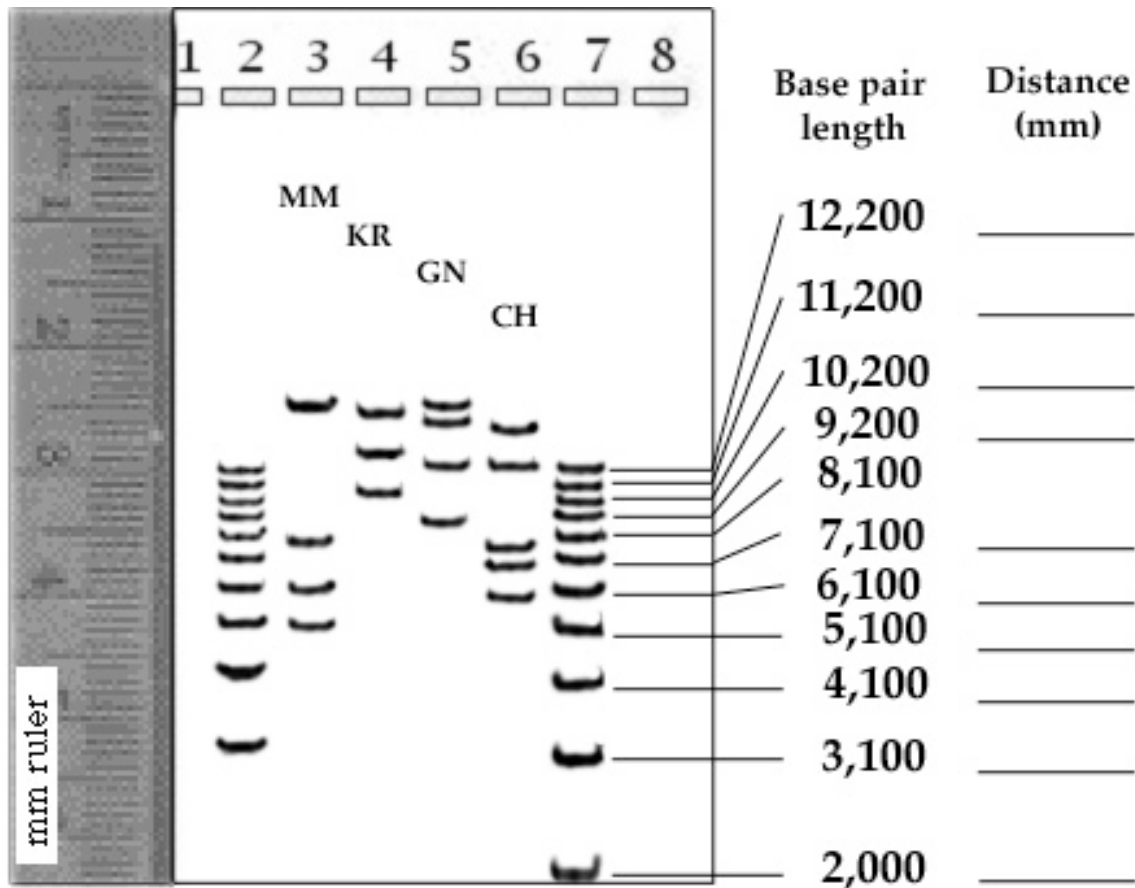
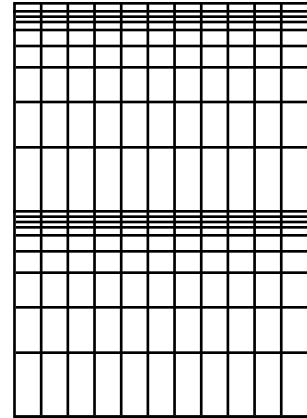


Figure 4. This agarose gel shows RFLP bands for one elephant from each of four African national parks. (MM= Masai Mara, KR=Kruger, GN=Gonarezhou, CH=Chobe). Marker IV 1kb+ is used as a ladder in lanes 2 and 7. Base pair lengths for the ladder are listed to the right of the gel.

1. For the ladder (lanes 2 or 7), measure the distance in millimeters from the well to each band. Use the magnified ruler in Figure 1 or a real millimeter ruler.
2. Record the distance measurements in the column at the far right of Figure 1.

3. Set up a graph on semi-log paper with 'distance traveled' (in millimeters) on the horizontal (X) axis and 'base-pair length' on the vertical (Y) axis. Orient the paper as shown at right. Label the axes.
4. Plot the distance and base-pair length data from the ladder lane onto the semi-log paper. Draw a straight line through the data. This 'standard line' should come as close as possible to all the data points.



5. For each DNA band in each lane, measure its distance (in millimeters) from the well. Use the standard line constructed from the marker lane to estimate as precisely as possible the sample band's length.
 - a. Enter the semi-log graph from the 'X' axis at a chosen distance traveled.
 - b. Move vertically on the graph until you intersect the standard line.
 - c. Move horizontally from the standard line until you intersect the Y axis.
 - d. Determine your base-pair length at that intersection with the Y axis.
6. Enter the distanced traveled and fragment length data for each sample below in Table I. Begin with the longest fragment as Fragment 1 and continue listing fragments in order of decreasing size. Note that not every sample will have the same number of RFLP fragments.

Table I. RFLP data for elephants from four national parks in Africa: distance traveled by each band (mm) and base-pair length (l).

Fragment	Masai Mara mm	l	Kruger mm	l	Gonarezhou mm	l	Chobe mm	l
1								
2								
3								
4								
5								
6								

TO ANALYZE YOUR OWN GEL, PLEASE DO THE FOLLOWING:

1. Record the length of each of the fragments of your known ladder or marker next to the appropriate band on the acetate sheet or drawing of your gel.
2. Generate a semi-log plot, same as was done for the Comstock gel (follow steps 1-5 above).
3. Record the lengths of the unknown bands from your gel on the Data Submission Form (DSF).

4. Compare the number and size of fragments from the ivory DNA to that of the Comstock gel database as well as to the samples you ran on your gel.
5. Record the most likely location or source of your ivory, based on DNA fingerprinting or banding patterns (fragment numbers and sizes) observed on your DSF.

DIRECTIONS FOR DATA SUBMISSION DS571

- Record the national park for the samples from Dr. Comstock's laboratory and the base-pair lengths of the RFLP fragments from Table I in list form in the upper portion of the handout called 'Data Submission Sheet.'
- Use the map of Africa you are given to find and record on the data submission sheet the countries in which the four parks are located.
- Using information from the teacher's overhead transparency, record the vegetation regime that prevails at each park (forest or savannah) on the Data Submission Form.

QUESTIONS TO FOLLOW ANALYSIS OF DATA

1. What causes RFLP patterns in lanes 3 to 6 of the Comstock gel to look different?

2. How are the RFLP patterns in lanes 3 to 6 of the Comstock gel similar? How are they different?

3. What are the two ways you have to compare RFLP data? What are the advantages and disadvantages of each? Which do you prefer?

4. Elephant populations from the four national parks represented in Dr. Comstock's data appear to have different RFLP patterns? Does this surprise you, or would you expect this? Why?

5. Why do you think that the RFLP patterns seem to be different between parks, but the same within parks?

6. Can you imagine situations in which more than one RFLP pattern might be found in a single park? How about situations in which one RFLP pattern might be found in more than one park?

7. Is knowing the RFLP pattern from one elephant in a park enough to conclude that all elephants in the park share the same pattern? Are two elephants enough? Three? How many would you test to decide that only one RFLP pattern is found in each park?

8. What risks to their survival do you think African elephants face today? Are all of the risks due to humans? What non-human risks might affect elephants' survival?

9. Hunting for ivory is not the only human activity that threatens the African elephant's survival. Clearing land for farming and harvesting trees decorative and insect-resistant lumber both affect elephant habitat. Which two populations of African elephants from your set of Dr. Comstock's samples are most at risk from logging and agricultural activity?

10. Do you think the risks for a park elephant in Africa are different from country to country?

DATE _____
 SUBMITTED BY _____

DATA SUBMISSION FORM – DS571
To: DR. KENINE COMSTOCK

Known Data:			Actual / Published
Sample	Country	Forest / Savannah	List of RFLP Lengths (bp)

New Data:			Actual / Published
Sample	Country	Forest / Savannah	List of RFLP Lengths (bp)



IDENTIFYING DNA CONCEPT QUESTIONS

ENZYME DIGEST CONCEPT QUESTIONS

1. What is an enzyme anyway?
2. What conditions influence how well an enzyme functions?
3. Do you think these conditions are also involved in how well a restriction enzyme functions? Why or why not.
4. Predict what would happen if the water bath or incubator were 100 degrees C?
5. What is the purpose for adding **restriction buffer** to the enzyme digest?
6. At what step or point in the lab will RFLP segments be formed? How are these fragments formed?
7. If populations of organisms have fragment lengths in common, what might that tell you about that population?

EXTENSION:

8. If offspring (children) result from the mating of two different RFLP populations, what would you predict their DNA fingerprint pattern might be?

GEL ELECTROPHORESIS CONCEPT QUESTIONS

1. What is a marker or ladder? Why is this considered a standard in this lab?
2. Why is a ladder or standard necessary part of this lab? Why does each lab team need to run their own ladder or standard?
3. How does the size of the DNA fragment affect its movement or migration through the agarose gel during electrophoresis?
4. Name three components found in the **sample loading buffer**. What is the purpose of each of these components?
5. Predict what would happen if you forgot to add the sample loading buffer?
6. Why do you add 1X TAE buffer to the gel box?
7. What would happen if you added water instead of the 1X TAE buffer and ran the gel with the water?
8. Why is uncut DNA included as a part of this lab?
9. How can you tell that the restriction enzyme digestion has occurred?
10. What is the purpose of placing the gel in Carolina Blu -2 (a stain) after the electrophoresis?
11. Predict, in your journal, what the gel will look like after it has been electrophoresed and stained with Carolina Blu-2.



VOCABULARY LIST WORKING DEFINITIONS

WHERE DID YOU USE THESE IN THE LAB AND WHY?

Agarose Gel	BamHI
Banding pattern	Biotechnology
Bromophenol blue	Buffer
Carolina Blu	Database
DNA Fingerprint	Enzyme digest
Gel electrophoresis	Genomic DNA
Ladder	Lambda DNA
Running Buffer	Marker
Micropipet	Mutation
Polymorphism	Recognition sequence
Recognition site	Restriction enzyme buffer
Restriction Enzyme	RFLP
Sample loading buffer	Standard
Uncut DNA	Unit of Activity
Xylene cyanol	



SCENARIO CONCLUSION

INSTRUCTIONS

1. Compare the base pair lengths of the restriction fragments from your first three samples (those from the National Parks) to those of Dr. Comstock to determine if there are any matches. What did you use as the basis for determining your matches? What conclusions can you draw from these two individual elephants?
2. If there are banding patterns (or fragment lengths) that don't match one of Dr. Comstock's samples, what conclusions can you make concerning these individual elephants?
3. Now examine your data from the ivory sample. What is the most likely location this ivory was obtained?
4. Can you be certain that the elephant from which the ivory was taken was from the location you identified? Why or why not?
5. Now that you have determined the countries of origin of each of the samples that you ran on your gel, what laws are in place concerning the trade of ivory in those countries? What are the laws in the United States regarding the importation of ivory?
6. Is your grandfather in trouble legally? Explain.
7. Summarize how biotechnology is used to establish a database and how this information (database) is used to help conserve a species.

